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THE EFFECT
OF DIET ON THE SURVIVAL OF
ADRENALECTOMIZED RATS

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

1935

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THE EFFECT OF DIET ON THE SURVIVAL OF ADRENALECTOMIZED RATS

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The physiology of the adrenal glands has been recently found to be intimately connected with salt metabolism. Cortical insufficiency is greatly ameliorated by sodium salts (cf. Gaunt, Tobin and Gaunt, 1935), while potassium salts exert a very noxious effect (Allers, Nilson and Kendall, 1936; Zwemer and Truszkowski, 1936). However, these have been, with exceptions to be discussed below, the only dietary factors to be explored in detail. Scott (1923) and Agate and Zwemer (1935) indicate that adequate diets are important variables in determining the total mortality of rats after adrenalectomy, but neither indicates precisely how. Accordingly an investigation was projected on the effects of various diets on the survival of adrenalectomized rats.¹

Cleghorn, Cleghorn, Forster, and McVicar (1936) obtained results, independently confirmed in this study, that indicate clearly that bread leads to a survival of adrenalectomized rats far longer than that secured with other diets. Typical of their results are the following: if rats were fed on meal cake only, 100 per cent of a group of 19 rats died within ten days; if bread was added to this diet, 43 per cent of a group of 17 rats died within 31 days.

Furthermore, it seemed possible that the reliability of the rat in studies of cortico-adrenal insufficiency might be dependent on the diets fed the animals. Variables known to affect the mortality of adrenalectomized rats are numerous. Colony differences in susceptibility, presumably due to accessory tissue, have been twice demonstrated by Gaunt (1933, 1935). Freed, Brownfield and Evans (1931) have suggested that careful removal of the pedicle along with the gland leads to high mortality. The conditions under which the animals are kept, particularly that of temperature have often been stressed as important. Agate and Zwemer (1935) consider meticulous asepsis important. In spite of these obstacles to consistent work, a large number of workers have recently reported high mortalities in the rat (cf. Gaunt, 1933; Agate and Zwemer, 1935), and

¹ Dr. B. O. Barnes suggested this problem. It is a pleasure to acknowledge his aid during its course. A preliminary report has been made (Swann, 1935).

many have used this animal in cortico-adrenal studies. Most agree on mortalities of about 90 per cent or greater. A vigorous dissent to such findings has recently been made by Agate and Zwemer (1935). They ascribe their low mortalities—only about 10 per cent—to the excellent living conditions and health of their rats and to the carefulness of the operation. They tested four colonies, all yielding about the same mortality.

METHODS. All the variables discussed above which are known to affect the mortality of adrenalectomized rats were kept as nearly as possible constant, the only varying factor being the diets fed the rats. Rats from the laboratory colony were used, with one exception which will be mentioned below. The operation was performed by the author, complete bilateral removal of the glands within their capsules along with the pedicles being made by the dorsal route. Ether anesthesia was employed. All rats, again with exceptions which will be discussed below, were 28 days of age.

The diets to be tested were fed to groups of 21 or more rats from weaning at 21 days of age until death ensued. At 28 days of age the operation was performed. After the operation, the animals were removed to a constant temperature room kept at 30°C. Records were kept of the sex, condition, operation, and period of survival of each animal. In some, daily weight and temperature changes were followed. Each litter of rats was so marked as to be identifiable. The rats were kept on clean sawdust in large cages; tap water was always available to the animals in large bowls.

RESULTS. The import of the data is best comprehended by means of graphs. In each of these, the cumulative percentage of deaths within a single dietary group is plotted against the number of days after adrenalectomy. From these, it can be appreciated when the animals were dying rapidly and what the incidence of death was within a single group. When such a curve is flat, it indicates that no deaths occurred; when steep, that a large portion of the group was dying. The percentage of a group dying on any given day can readily be computed by finding the difference between the percentage dead at the beginning and at the end of the day.

The following diets were tested:

No. 1	No. 2
67% cracked corn 33% powdered skim milk 21% fresh whole milk 59% bread 4% raw beef lung 16% water Lettuce } ad lib. About 10 grams per rat per day	60% fresh whole milk 40% bread 1.46 mgm. FeCl_3 0.013 mgm. CuSO_4 Lettuce } Per rat per day

No. 3	No. 9. Sherman "optimal" (1932)
Bread, dried and ground fine	67% ground whole wheat flour
Lettuce	33% powdered whole milk
No. 4	Lettuce
77% starch	No. 10. Scott diet (1932)
14% casein	60% hominy
5.8% dried yeast	15% rolled oats
3.2% Osborne and Mendel salt mixture	14% dried meat scrap
Lettuce	10% powdered whole milk
No. 5	1% salt
75% starch	Lettuce
14% casein	No. 11. Agate and Zwemer diet (1935)
5.8% dried yeast	37.5% yellow corn meal
5.2% O. and M. salt mixture	37.5% ground whole wheat
Lettuce	16% linseed oil meal
No. 6	5% casein
"Fox-chow"—Ralston Purina Co.	2% ground alfalfa
Lettuce	1.5% bone ash
No. 7	0.5% NaCl
50% butter	67.5% ground whole wheat
14% lard	15% casein
25% casein	10% whole milk powder
5% cod liver oil	5% butter
6% O. and M. salt mixture	1.5% CaCO ₃
Lettuce	1% NaCl
No. 8	Lettuce and meat scraps
91% casein	No. 12
5.8% dried yeast	"Pel-ets"—Nowak Milling Co.
3.2% O. and M. salt mixture	Lettuce
Lettuce	

Diets 4, 5, 7 and 8 are each of a special type. No. 4 (Osborne and Mendel, 1920; Levine and Smith, 1927) is high in carbohydrate but low in fat. No. 7 (Osborne and Mendel, 1921a; Levine and Smith, 1927) is high in fat, but low in carbohydrate. No. 8 (Osborne and Mendel, 1921b) is high in protein but low in fat and carbohydrate. No. 5 is high in salts.

Diets 10 and 11 have been used by workers reporting very low mortalities in the rat after adrenalectomy. Diets 6 and 12 are commercial diets. No. 12—"Pel-ets"—is meant for rabbits, not rats, but it has been successfully used with mature rats. Number 6 has been found satisfactory for rats of any age.

An effort was made, no. 3 excepted, to have all these diets adequate. Except for no. 3, all have been reported adequate for mature rats. Our 28-day rats however were, in the case of some diets, not in a state of

good nutrition when they came to operation. The offending diets on this point were nos. 3, 7, 8 and 12; they cannot, therefore, be considered adequate diets. Also no. 4 has been found inadequate for growth over a long period; for a month, however, even these young rats do well on it. All the rest of the diets were thought to be satisfactory.

In figures 1 and 2 the mortality rates of each group of animals on these diets are presented, along with the number of animals tested. Each of these rates falls into one of two types: the one has an early and rapid death rate, 70 per cent or more of the deaths in all groups having occurred by the ninth day after adrenalectomy and 95 per cent or more by the twenty-first day. Diets 4 through 12 yield this type of mortality curve.

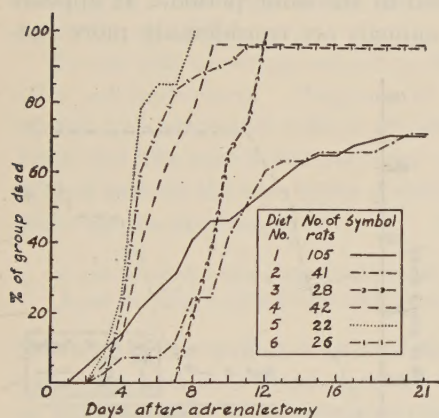


Fig. 1

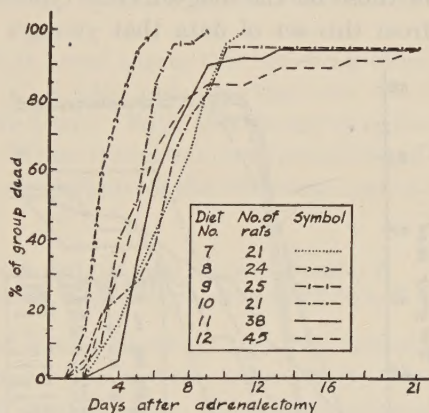


Fig. 2

This type of curve will subsequently be referred to as a "short-survival" mortality curve.

The second type of curve—to be called the "long-survival" type—has a less rapid rate: by the ninth day after adrenalectomy about 30 per cent have died and by the twenty-first day an average of 75 per cent. Diets 1, 2 and 3 are of this type. The median death of the former group occurs on the fifth day, that of the latter group on about the ninth day—an average survival by the latter group almost twice that of the former.

Although there is considerable variation in each of these two general types of curve, the data are not considered adequate for any attempt to find a significant difference between any of the diets in either of the two main groups. More animals would be needed to settle such a difference, if it exists.

It seemed desirable to ascertain whether other colonies would react similarly to this dietary difference in susceptibility to adrenalectomy. Four colonies were tested. The number of rats used and the results are

presented in figure 3. Stock I consisted of albinos from the laboratory colony. Stock II consisted of mixed rats from the Department of Psychology, University of Chicago. Stock III and stock IV, albinos and McCollum type rats respectively, were obtained from different dealers. No colony difference was found, each colony yielding mortality curves similar to the two types obtained with the main test colony.

The two types of diet were tested on mature rats, no. 1 as representative of long-survival diets and no. 4 as representative of short-survival diets. In figure 4 the results are shown. The difference in the mortality rate of mature rats is marked, 90 per cent of those on the short-survival type of diet having died by the twenty-first day whereas 13 per cent of those on the long-survival type died in the same period. It appears from this set of data that younger animals are considerably more sus-

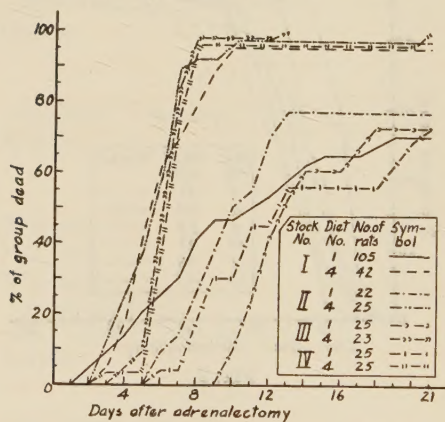


Fig. 3

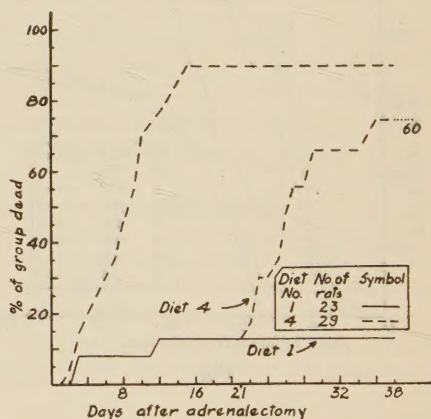


Fig. 4

ceptible to adrenalectomy on either type of diet. Mature rats on the long-survival type of diet survive the operation as a group quite well, maintaining their weight and, apparently, good health.

In order further to prove that it is a dietary effect which leads to this difference in mortality rates, the survivors of groups on diets 1 or 2 were placed at 21 days after adrenalectomy on a short-survival type of diet, i.e., on no. 4. Controls were also made to determine how the mortality rate of rats kept on the survival type of diet changed subsequent to 21 days after operation. Figure 4 shows that while no mature rats died during the fourth week after adrenalectomy if kept on the long-survival type of diet, most of them died in about this same period if changed to the short-survival type of diet. The same holds true for young rats. A group of them so treated showed a final mortality of 97 per cent.

DISCUSSION. That the long-survival type of diet is by no means a

complete replacement therapy is indicated by a variety of facts: in the first place, 75 per cent of young animals and 18 per cent of old die under this treatment, indicating that it is not adequate replacement for all animals. Secondly, the growth of 28-day adrenalectomized rats on long-survival diets is much retarded. At 60 days of age, 32 days after adrenalectomy, they averaged 111 grams, about 30 per cent underweight.

The common factors in diets 1 and 2, both long-survival types of diet, are bread and milk. When bread alone is fed (diet 3), the effect of the operation is delayed. The rats do not die until 7 days after operation, but then deaths in the group occur rapidly until on the twelfth day, 100 per cent have died (fig. 2). This is somewhat different from the long-survival type of curve; possibly the relative adequacy of the diets will account for the difference.

We are still uncertain why it is that bread exerts this beneficial effect after adrenalectomy. Cleghorn et al. (1936) consider that the effect cannot be ascribed to sodium chloride alone. With this we are in agreement, but the case is not yet clear. When the sodium and potassium intake of rats on the two types of diet are measured, the following approximate figures are found:

A rat on diet 3 (long-survival) consumes daily 74 mgm. Na and 20 mgm. K

A rat on diet 6 (short-survival) consumes daily 15 mgm. Na and 44 mgm. K

Not only is the sodium intake of diet 3 comparatively high, but also the potassium intake is comparatively low. The reverse is true in the case of both cations in a short-survival type of diet. Because in adrenal insufficiency sodium exerts a beneficent effect, but potassium a noxious effect, the effect of these diets may be due to their sodium and potassium contents. Indeed, when the sodium intake of rats on diet 6 (short-survival type) was raised to 74 milligrams by adding sodium chloride to the drinking water, a group of 24 rats died at the long-survival rate.

Both of these facts might seem to indicate that the beneficent effect of bread is ascribable to its relative sodium and potassium content. However, we have found that salt will not produce such effects in the short-survival types of diet unless the salt is in the drinking water, i.e., salt did not prolong the life of a group of 31 rats when added dry to dry diet no. 6. Apparently it is a matter of fluid balance as well as salt and other balances.

Two of the diets here tested have been used by other workers, each reporting low mortalities after adrenalectomy. These diets are nos. 10 and 11, those of Scott (1923) and Agate and Zwemer (1935); both of them yielded mortalities of 95 per cent in our hands. Whatever may be the cause of the discrepancy in results, it does not appear to be the diets employed.

When the odd 25 per cent of rats surviving all diets (cf. fig. 4) were brought to autopsy, there were almost invariably found at the head of the kidney large pieces of adrenal tissue, often of the size of a normal gland. Consequently we have been able to ascribe such long survivals to these pieces, and not to an abnormal resistance of the rat to adrenal ablation. Such pieces are presumably due to hypertrophied tissue, resulting either from accessory tissue or incomplete removal of the gland.

It has also been found that the effect of bread-containing diets on adrenalectomized rats cannot be duplicated on dogs. Three dogs, well habituated to a diet of bread and milk, were adrenalectomized in a two-stage operation. They died, respectively, in 2, 2 and 4 days, with controls on meat diets dying in about the same period after the second operation was performed.

SUMMARY

The susceptibility of groups of about 25 28-day rats to adrenalectomy has been found to be quite constant on a number of diets, including the Sherman "Optimal," "Fox-chow," the Agate and Zwemer combination (1935) and others. Mortalities of 95 per cent or greater were obtained, with the median death occurring on about the fifth day after operation. However, one substance, bread, leads to both a prolongation of life and a smaller total mortality. About 72 per cent of rats on bread-containing diet succumb to the operation, with the median death occurring on the ninth day.

Mature rats show a clearer difference in this dietary effect. Whereas 13 per cent die on a bread-containing diet, 90 per cent die on a bread-less diet.

Four colonies were tested, all yielding similar results. In all, 704 rats have been employed.

When those rats which had survived on bread-containing diets for 21 days were changed to bread-less diets, they died within a week. This is taken as a further indication that the differences in mortality rates are due to dietary variables.

Bread will not, however, lengthen the life of adrenalectomized dogs.

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